MAPPING RIBO-SEQ AND RNA-SEQ SAMPLES to GENOME in RIBOGALAXY

This is the third part of the tutorial. Here the user can learn how to map Ribo-Seq and matching RNA-Seq reads to a genome and prepare files that can be transferred to GWIPs-viz and visualised there using the RiboGalaxy instance available at https://ribogalaxy.genomicsdatascience.ie/. At the end of the tutorial the user will be able to see the alignments in the genome context.

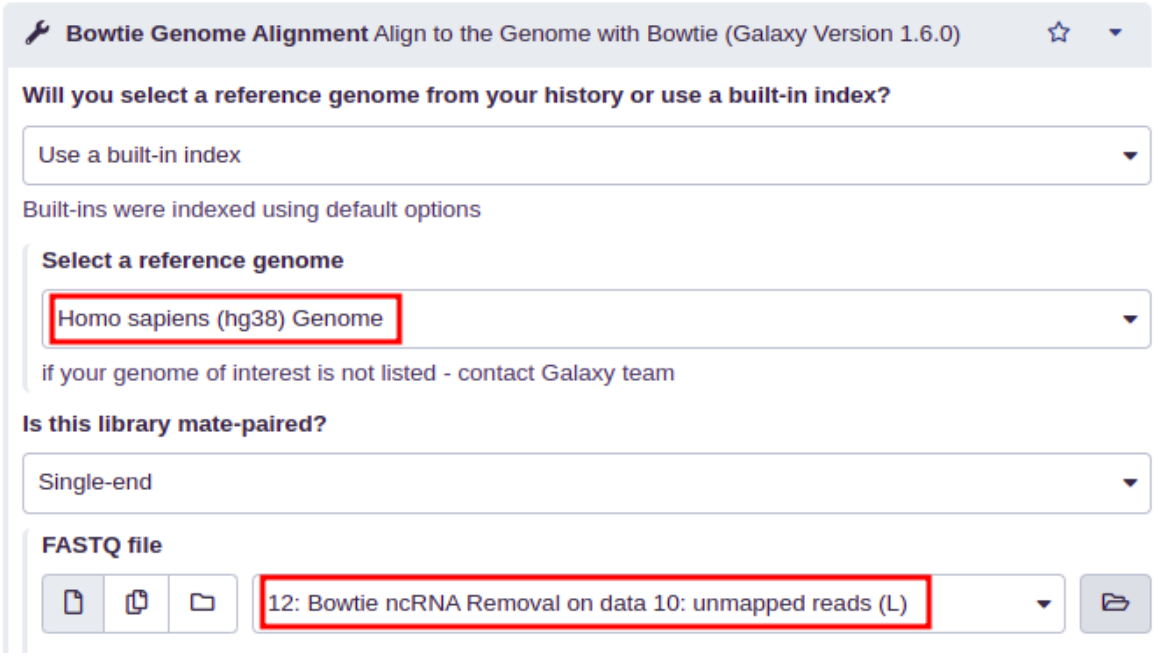
As input, you will need clean FASTQ files from part 1 of the tutorial (no adapters, UMIs, untemplated additions, barcodes and ncRNA contaminants). We assume here that you have the files in the RiboGalaxy instance and we are going to continue working with it.

Mapping RIBO-seq to a genome

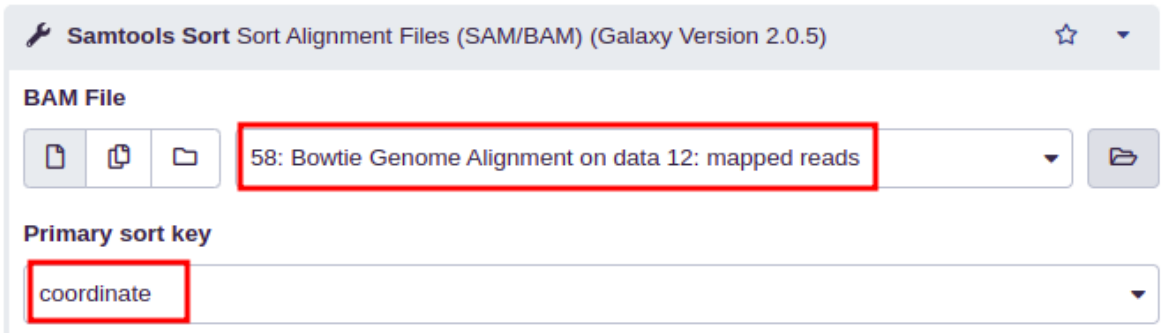
Let's briefly go through the main steps.

1. First we will map clean reads (ribosome protected fragments) to the genome using ***Bowtie Genome Alignment***.
2. Then we sort alignments based on coordinates using ***Samtools sort***.
3. Next we will deduplicate a sample based on UMIs using ***UMI-tools deduplicate***.
4. Then we again sort alignments based on coordinates using Samtools sort.
5. We need to obtain chromosome sizes by using the **Get *Chromosome Sizes*** tool.
6. Using the deduplicated bam file from step 4, now we can create ribosome profile in bed format using ***Create Ribosome Profiles*** tool.
7. Next we will convert the bed file to BigWig so that it can be uploaded and visualised in GWIPS-viz by using ***Convert a BED File to a BigWig*** tool.
8. In order to upload the resulting BigWig track file to GWIPS-viz, we will need to create a link using the Generate **Custom Track** tool.

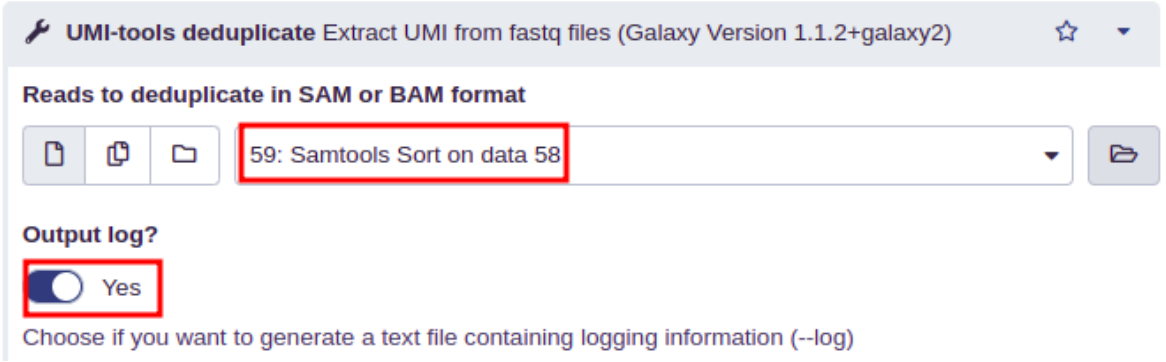
1. Map reads (RPFs) that were cleaned from rRNA/tRNA contamination (after the last step of STAGE I) on the genome using ***Bowtie Genome Alignment*** in **GWIPS-Viz (genomic alignment) branch**. You can choose the built-in index *Homo sapiens (hg38) Genome*. Click 'Execute'.



2. Sort the resulting bam file by coordinate using ***Samtools sort*** from **GWIPS-Viz (genomic alignment) branch**. Click 'Execute'.



3. This step can be performed if you want to deduplicate your sample (remove PCR duplicates) using UMIs. It can be done with ***UMI-tools deduplicate*** from **UMI and barcodes**. Input file is bam sorted by coordinate.You can also choose to output log file. Click 'Execute'.

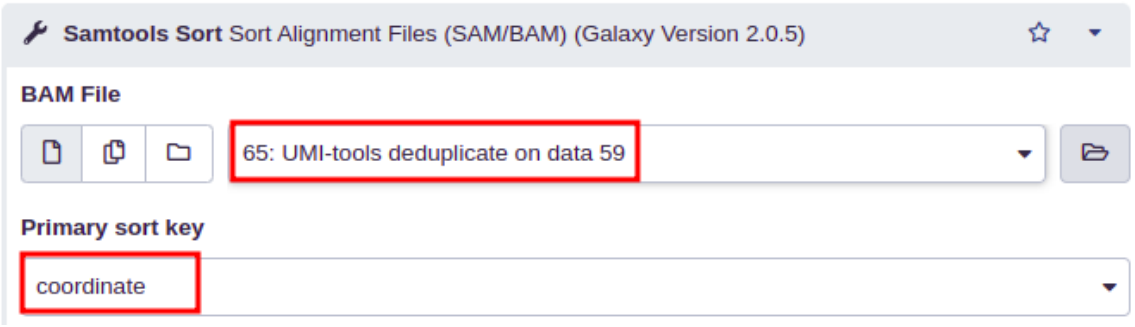


You can see in the log file that only 3639 reads were in input and 3403 are left after deduplication. The original sample had about 100k reads. This significant downsampling happened because most of the reads were multimappers (remember

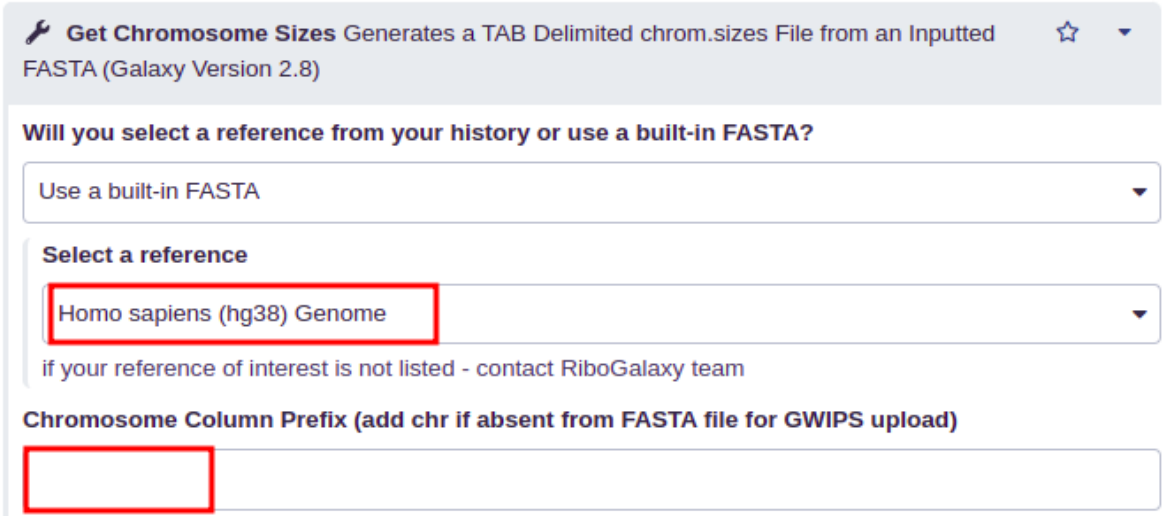
that we had to tick ‘Allow ambiguously mapped reads’ in part 2 of the tutorial?) and when we prepare samples for GWIPs-viz, we remove multimappers.

```
2023-02-04 15:24:03,797 INFO Reads: Input Reads: 3639
2023-02-04 15:24:03,797 INFO Number of reads out: 3403
```

4. Sort the resulting deduplicated bam file by coordinate using ***Samtools sort*** from **GWIPS-Viz (genomic alignment) branch**. Click ‘Execute’.

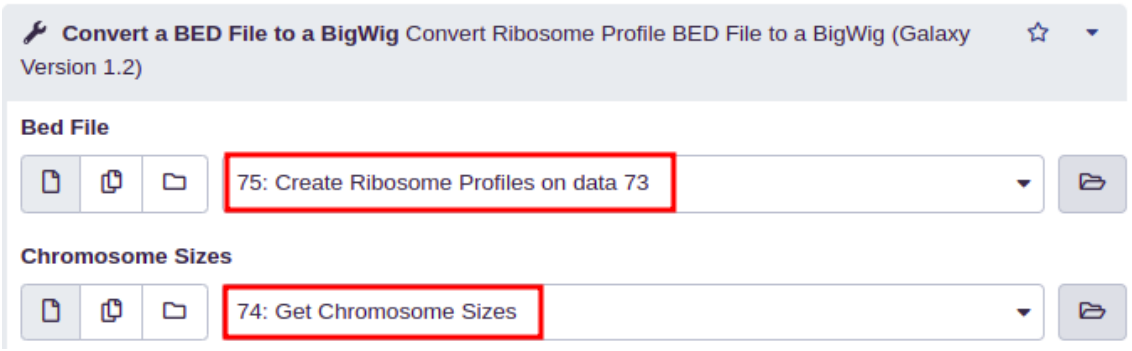


5. Now we will get chromosome sizes for subsequent transformation of alignment to the ribosome profile. We will use the ***Get Chromosome Sizes*** tool from **GWIPS-Viz (genomic alignment) branch**. Select built-in fasta, Homo sapiens (hg38) Genome. Click ‘Execute’.

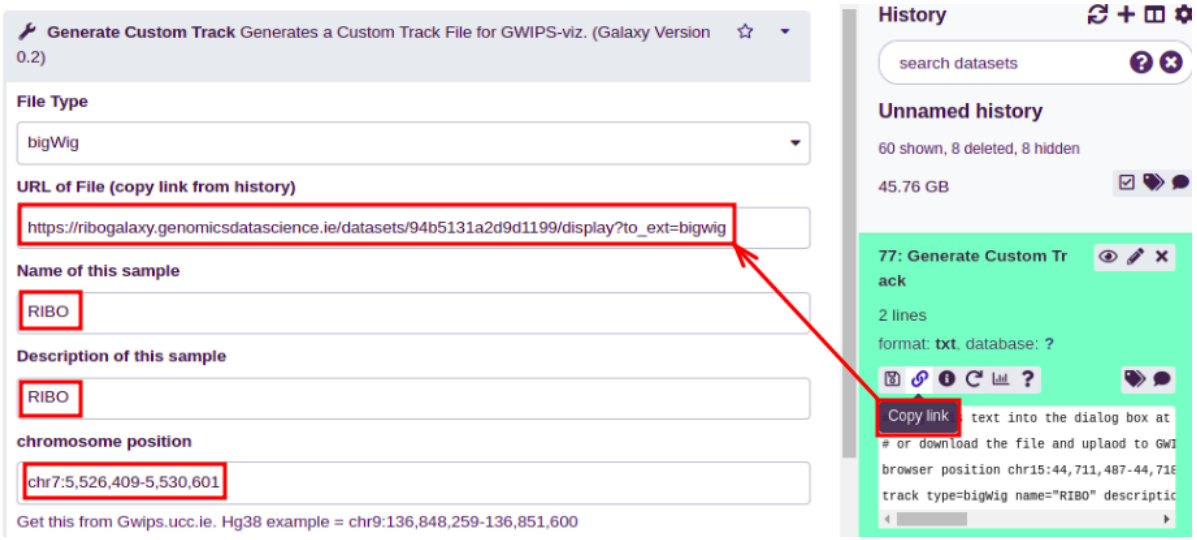


6. Create ribosome profile in bed format from sorted by coordinate deduplicated bam file from step 4 using ***Create Ribosome Profiles*** tool in **GWIPS-Viz (genomic alignment) branch**. Choose built-in fasta, *Homo sapiens (hg38) Genome*. Click ‘Execute’.

7. Next we need to convert the bed file from step 6 to BigWig file. BigWig can be uploaded to genome browsers, e.g. GWIPS-viz. We will use the ***Convert a BED File to a BigWig*** tool from **GWIPS-Viz (genomic alignment) branch**. Another input is chromosome sizes file from step 3. Click 'Execute'.

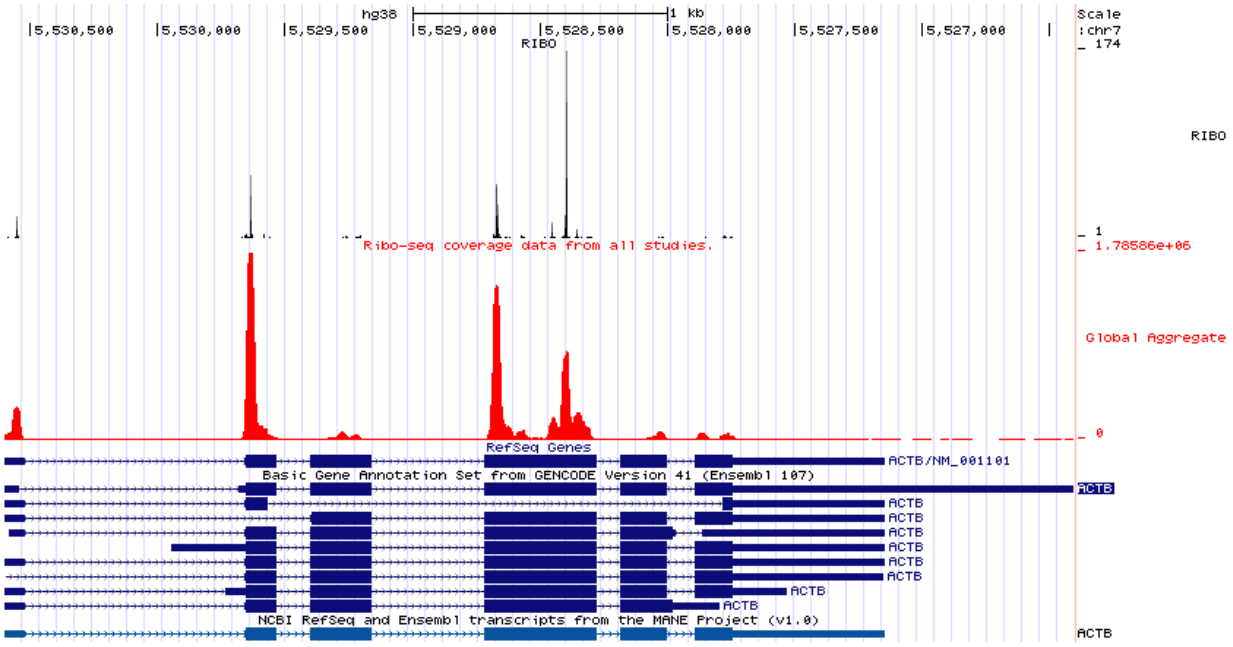


8. Finally, we can generate the file for easy upload of the BigWig file generated on step 7 to the GWIPS-viz browser. We will use **Generate Custom Track** from **GWIPS-Viz (genomic alignment) branch**. You will need to copy a link to the BigWig file and use it as input. Add name and description of the sample, as well as any chromosome position of interest, e.g. chr7:5,526,409-5,530,601 (it can be changed in genome browser). Click 'Execute'. This tool will output a file containing a link. You need to download this file and then upload to the GWIPS-viz browser.



In order to upload this file, go to https://gwips.ucc.ie/cgi-bin/hgGateway, select **My Data**, **Custom Tracks** and **add custom track. Choose file** (or copy and paste its content) and **submit**.

You then can take a look at the data. Depending on your browser configurations, you will see different tracks. One of them should be the track ‘RIBO’ (you can right click on that and choose ‘full’ to see its expanded version). Also we enabled ‘Global Aggregate’ of Elongating Ribosomes (A-sites), track coloured in red. You can see here that there are similarities between these tracks.

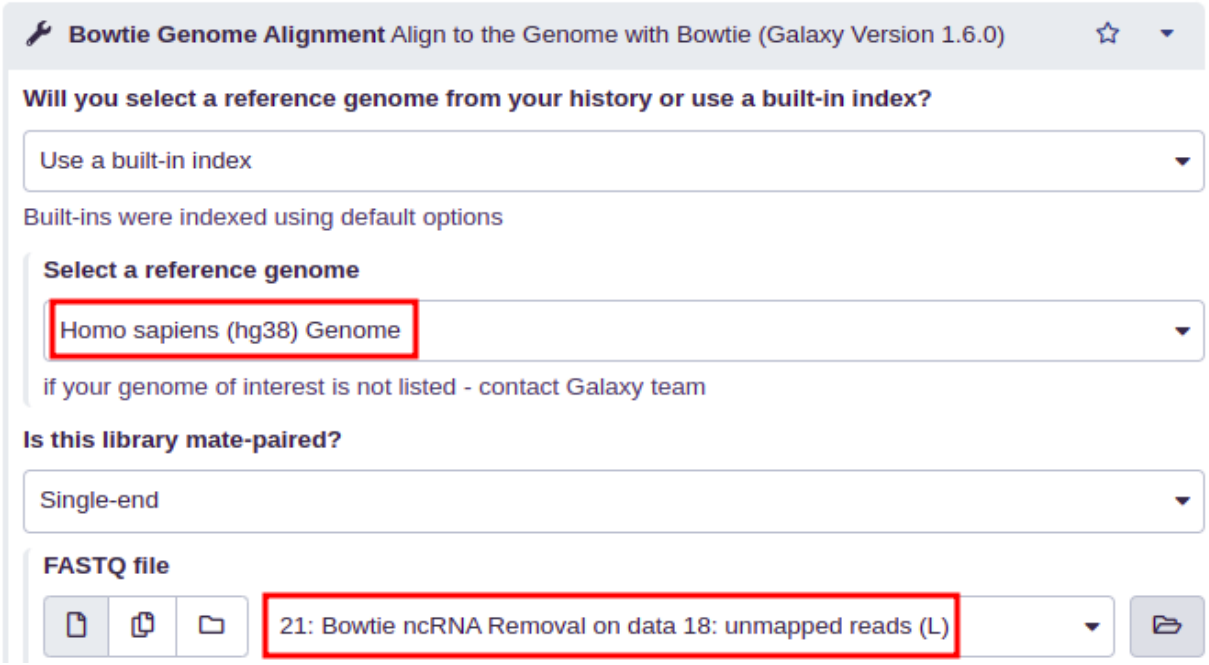


Mapping RNA-seq to a genome

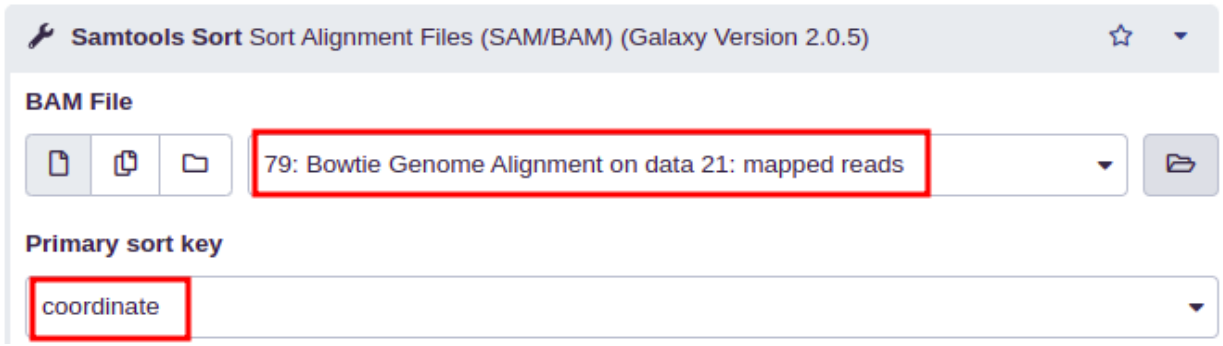
The main steps are similar to the Ribo-Seq sample except for some last steps.

1. First we will map clean reads (ribosome protected fragments) to the genome using ***Bowtie Genome Alignment***.
2. Then we sort alignments based on coordinates using ***Samtools sort***.
3. We need to obtain chromosome sizes by using the Get ***Chromosome Sizes tool***.
4. Using the sorted BAM file from step 2, we will create a coverage file in BED format using **BedTools Genome Coverage.**
5. Next we will convert the BED file to BigWig so that it can be uploaded and visualised in GWIPS-viz by using ***Convert a BED File to a BigWig*** tool***.***
6. In order to upload the resulting BigWig track file to GWIPS-viz, we will need to create a link using the Generate **Custom Track** tool.

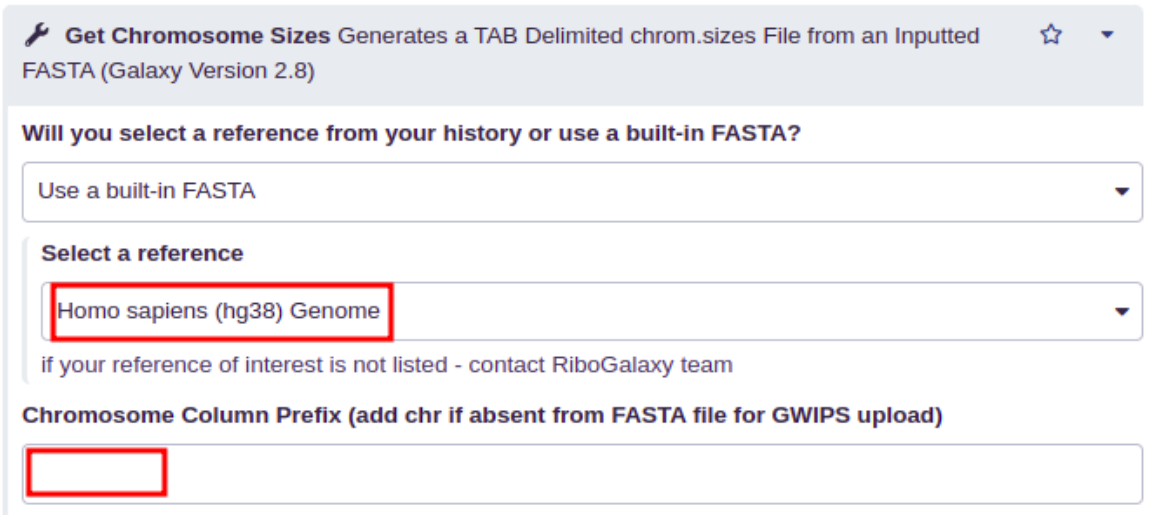
1. Map clean RNA-seq reads to the genome using ***Bowtie Genome Alignment*** in **GWIPS-Viz (genomic alignment) branch**. You can choose the built-in index *Homo sapiens (hg38) Genome*. Click 'Execute'.



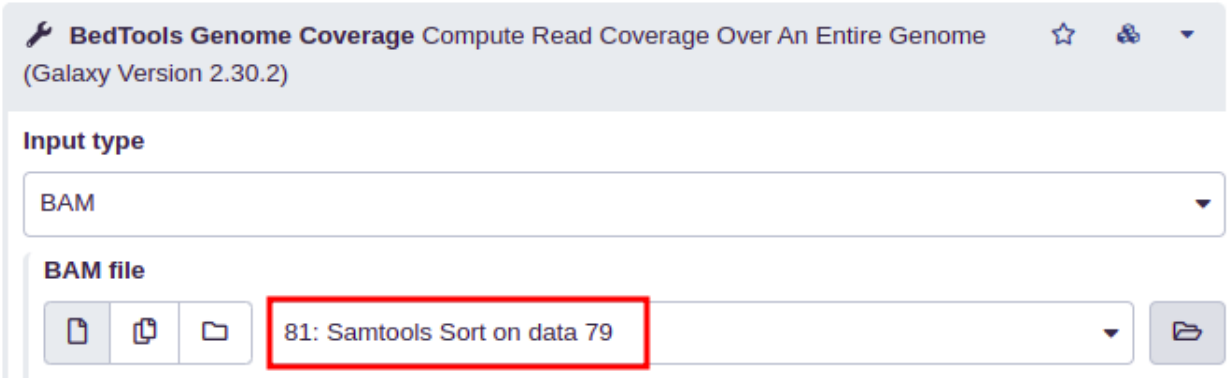
2. Sort the resulting bam file by coordinate using ***Samtools sort*** from **GWIPS-Viz (genomic alignment) branch**. Click 'Execute'.



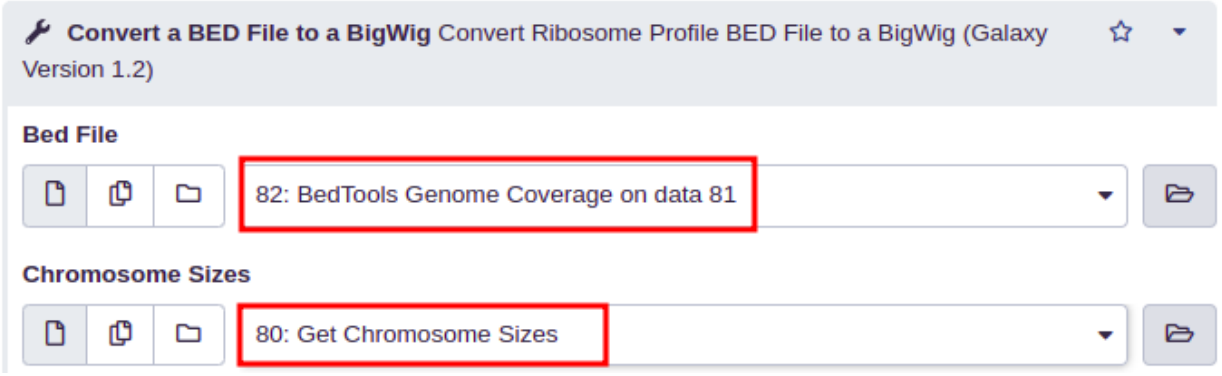
3. Now we will get chromosome sizes for subsequent transformation of alignment to the ribosome profile. We will use the ***Get Chromosome Sizes*** tool from **GWIPS-Viz (genomic alignment) branch**. Select built-in fasta, Homo sapiens (hg38) Genome. Click 'Execute'.



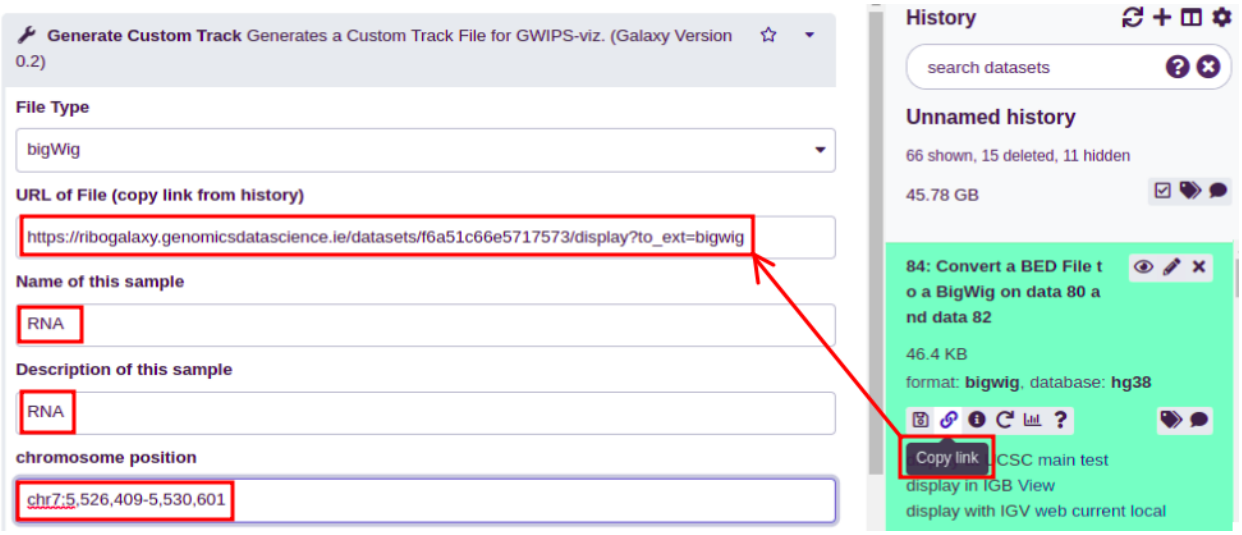
4. Create coverage file in BED format from sorted by coordinate BAM file from step 2 using **BedTools Genome Coverage** tool in **GWIPS-Viz (genomic alignment) branch**. Choose built-in fasta, *Homo sapiens (hg38) Genome*. Click 'Execute'.



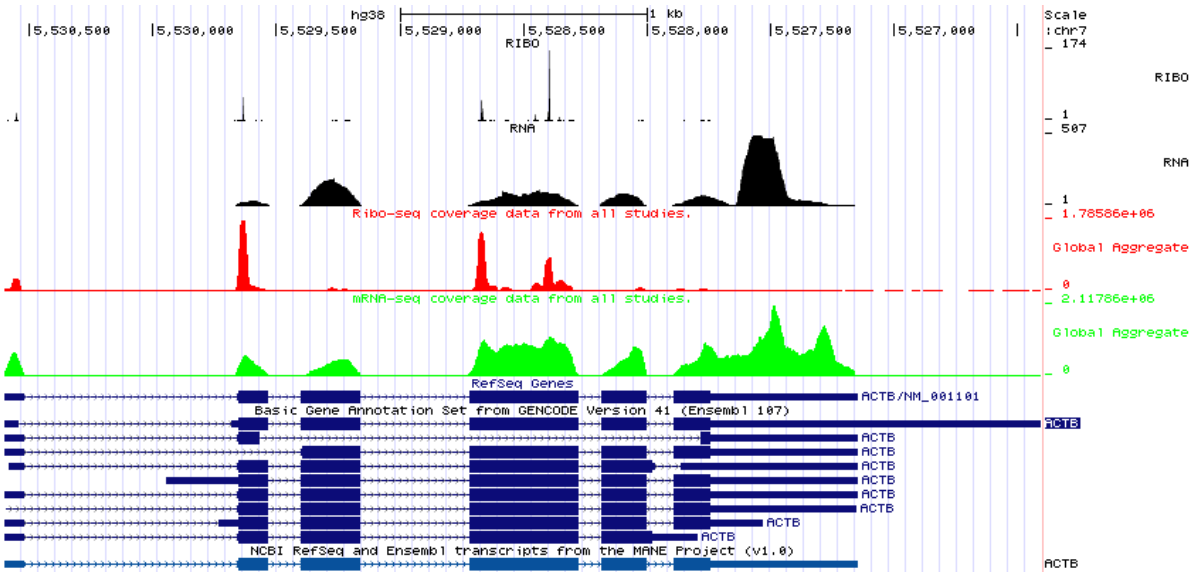
5. Next we need to convert the bed file from step 4 to BigWig file. BigWig can be uploaded to genome browsers, e.g. GWIPS-viz. We will use the ***Convert a BED File to a BigWig*** tool from **GWIPS-Viz (genomic alignment) branch**. Another input is chromosome sizes file from step 3. Click 'Execute'.



6. Finally, we can transform the BigWig file from the previous step for easy upload to the GWIPS-viz browser. We will use **Generate Custom Track** from **GWIPS-Viz (genomic alignment) branch**. You will need to copy a link to the BigWig file and use it as input. Add name and description of the sample, as well as any chromosome position of interest, e.g. chr7:5,526,409-5,530,601 (it can be changed in genome browser). Click 'Execute'. This tool will output a file containing a link. You need to download this file and then upload to the GWIPS-viz browser.

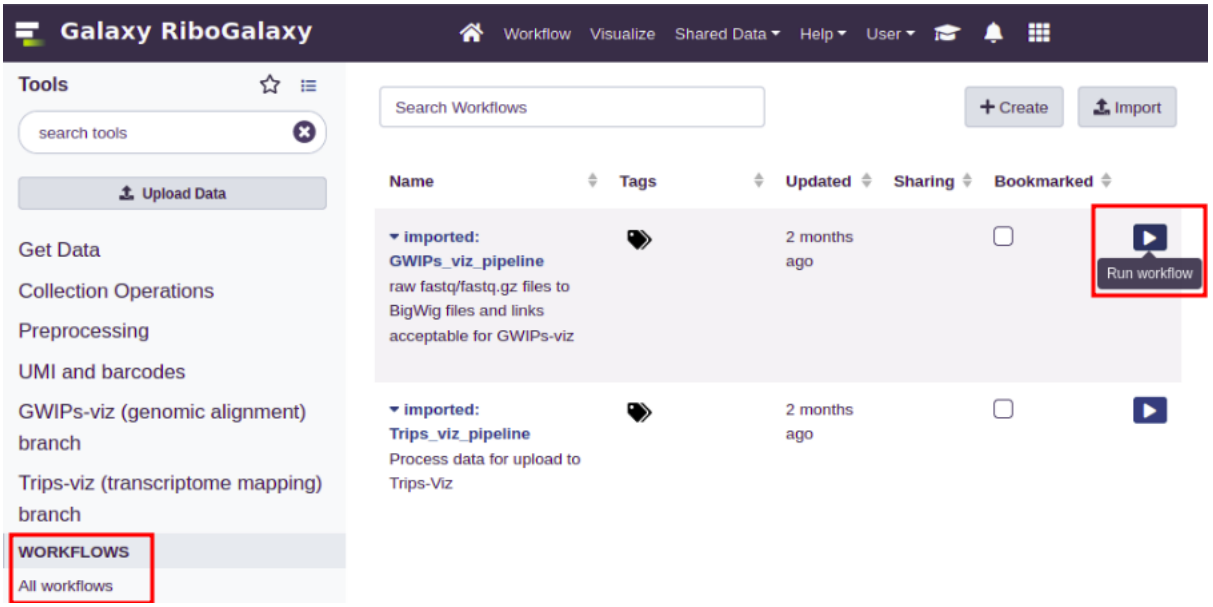


In order to upload this file, go to https://gwips.ucc.ie/cgi-bin/hgGateway, select **My Data**, **Custom Tracks** and **add custom track. Choose the file** (or paste its content) and **submit**. You then can take a look at the data. Depending on your browser configurations, you will see different tracks. One of them should be the track 'RIBO', another one is 'RNA'. Also we enabled 'Global Aggregate' of Elongating Ribosomes (A-sites), track coloured in red and 'Global Aggregate' of RNA-seq reads, track is green. You can see here that there are similarities between RNA-seq aggregate and our uploaded 'RNA' track.

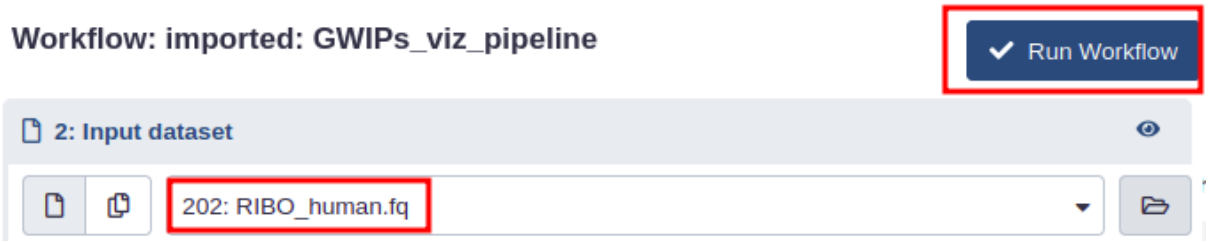


Workflow for Ribo-Seq sample

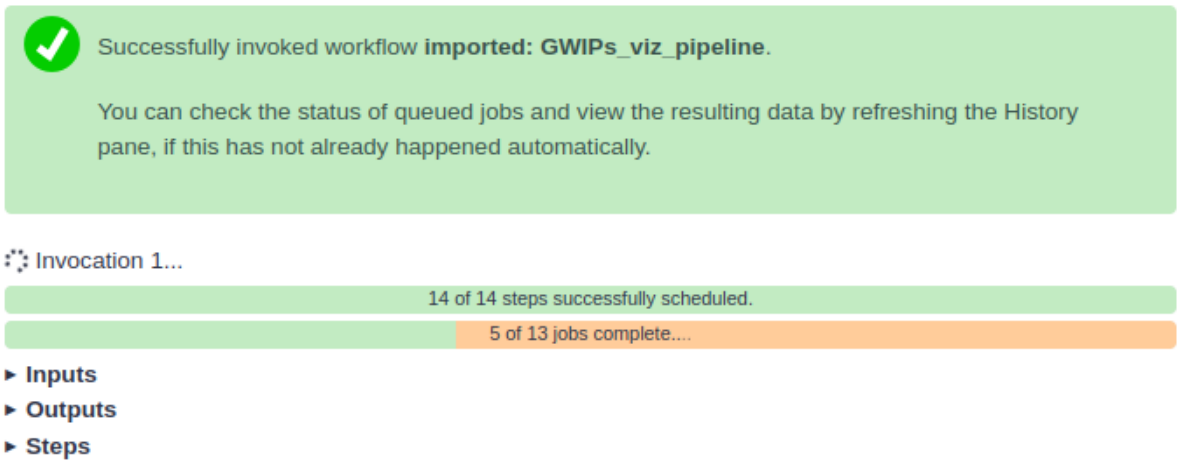
For the Ribo-seq sample, we also created a Workflow which was tailored (all the parameters are set) to process the particular read structure from raw data to BED file. Generation of file compatible with GWIPs-viz requires an extra step. First, you need to choose ‘All workflows’ on the left panel and then select ‘Run workflow’ for GWIPs_viz_pipleine.



Next you need to make sure that the input file is correct and hit ‘Run Workflow’.

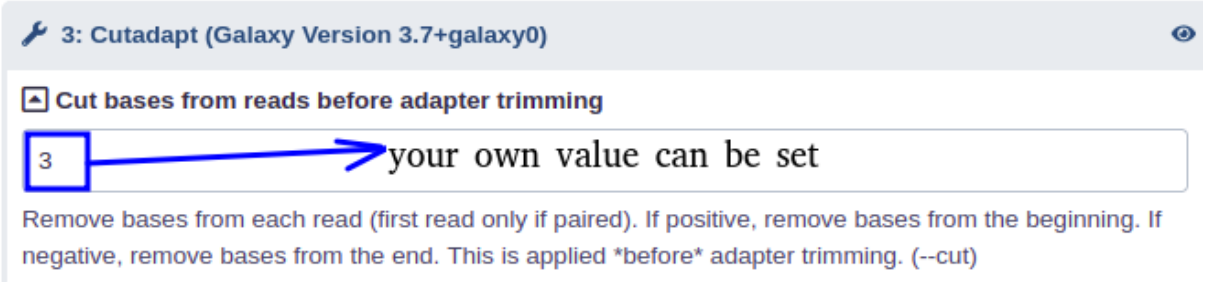


Now can can just monitor the progress of the Workflow:



Once it’s finished, you will have a BED file and you can use **Generate Custom Track** from **GWIPS-Viz (genomic alignment) branch**.

The workflow parameters are customisable:



You can also create your own workflows.